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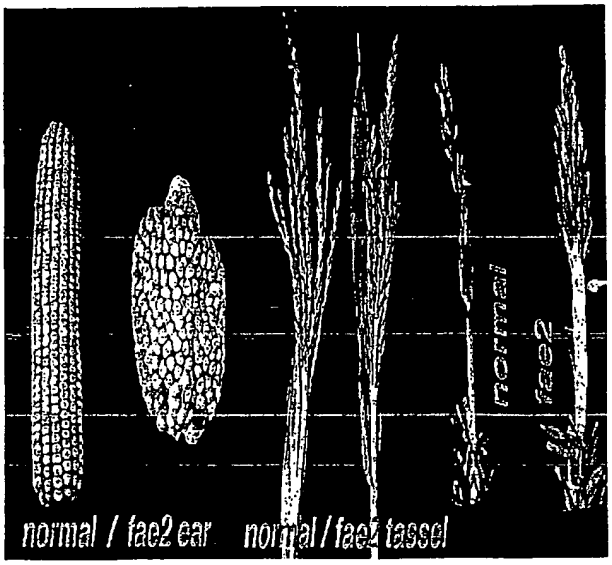
(54) Title: NOVEL INFLORESCENCE MERISTEM DEVELOPMENT GENE, PROMOTER, AND METHODS OF USE FOR SAME



(57) Abstract: The invention relates to the isolation and characterization of a novel maize gene (fae2) responsible for meristem proliferation and inflorescence development. The novel gene, gene product, and regulatory regions may be used to manipulate meristem growth, inflorescence development and arrangement, and ultimately to improve yield of plants. The invention includes the novel gene and protein product as well as the use of the same for temporal and spatial expression in transgenic plants to enhance kernel development, alter plant morphology and increase yield in plants.

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TITLE: NOVEL INFLORESCENCE MERISTEM DEVELOPMENT GENE, PROMOTER, AND METHODS OF USE FOR SAME

FIELD OF THE INVENTION

This invention relates generally to the field of plant molecular biology. More specifically, this invention relates to the isolation and characterization of a novel gene responsible for meristem proliferation and inflorescence development. The novel gene, gene product, and regulatory regions may be used to manipulate meristem growth, inflorescence development and arrangement, and ultimately yield of plants.

BACKGROUND OF THE INVENTION

Plant development is highly dynamic and involves the controlled and organized initiation of new primordia from groups of stem cells called meristems. Development of leaves, axillary meristems and flowers are initiated in regular patterns from the shoot apical meristem (SAM). SAMs show remarkable abilities to regulate their size during development, by balancing cell proliferation with the incorporation of cells into new primordia (Sussex, 1952; Medford, 1992; Weigel and Clark, 1996; Meyerowitz, 1997).

The understanding of SAM function has increased significantly in recent years, especially from analysis in model plants such as *Arabidopsis* (Bowman and Eshed, 2000; Evans and Barton, 1997). Mutants affecting the SAM can be grouped into two classes.

The first class involves mutations affecting shoot meristem initiation and/or maintenance. In strong alleles of *shoot meristemless* (*stm*), the shoot meristem fails to form or to be maintained (Barton and Poethig, 1993), and weaker alleles indicate a role throughout all stages of meristem development (Clark et al., 1996; Endrizzi et al., 1996). *STM* encodes a *knotted1* (*kn1*) related homeodomain protein; these genes are expressed throughout the meristem but down regulated on the flanks where organs are initiated

(Jackson et al., 1994; Long et al., 1996; Smith et al., 1992). *wuschel* (*wus*) mutants are also impaired in stem cell maintenance (Laux et al., 1996). *WUS* encodes a homeodomain protein that is expressed prior to the appearance of the SAM in the embryo, and becomes localized to a group of cells that underlies the presumptive stem cells, suggesting it may signal stem cell fate non-autonomously (Mayer et al., 1998).

The second class involves mutations affecting meristem proliferation and fasciation. Fasciations, from Latin fascis, meaning bundle, are variations in plant form resulting from proliferative growth. Fasciation was one of Mendel's 14 heritable characters in pea. (Emerson, 1912). Fasciated mutants have significantly increased crop yields, for instance by increasing tomato fruit size (Luckwill, 1943; Zielinski, 1945). Mutations in *Arabidopsis* that cause enlargement of the shoot apex and frequently fasciation include the *clavata* mutations (Leyser and Furner, 1992; Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998), *mgoun1* and *2* (Laufs et al., 1998) *fasciata1* and *2* (Leyser and Furner, 1992) and *fully fasciated* (Medford et al., 1992). *clv* mutations develop club shaped siliques and have larger shoot meristems and increased floral organ number. *clv1* and *3* strong alleles often fasciate (Clark et al., 1993; Clark et al., 1995), and double mutants resemble either single mutant. Furthermore, *clv3* can dominantly enhance *clv1* mutants, suggesting these genes act together in the same pathway (Clark et al., 1995). *clv2* mutants on the other hand show relatively weak phenotypes; similar to weak *clv1* or *clv3* alleles, and show additional phenotypes in pedicel, valve and stamen development. Fasciation of *clv2* mutants is observed only rarely, and only under short day growth conditions, which may indicate genetic redundancy (Kayes and Clark, 1998). Whilst *clv1* and *clv3* strong alleles are epistatic to *clv2*, *clv2* enhances weak or intermediate alleles of *clv1* or *clv3*, suggesting these genes act in the same pathway to regulate shoot meristem size (Kayes and Clark, 1998).

The two classes of mutations interact. For example, *stm* mutants dominantly suppress *clv* mutants, and *clv1* and *clv3* dominantly suppress *stm*,

suggesting they act in competing pathways to regulate shoot meristem proliferation (Clark et al., 1996; Kayes and Clark, 1998). *clv* and *wus* mutants also display molecular and genetic interactions which suggest they act in a regulatory feedback loop to control the size of the stem cell population (Brand et al., 2000; Schoof et al., 2000).

The specification of stem cells and control of their proliferation is fundamental to all organisms and of relevance to diseases such as cancer as well as aging. Despite recent advances in understanding the mechanism of stem cell proliferation in *Arabidopsis*, relatively little is known about how well these mechanisms are conserved throughout the plant kingdom, or how they affect the development of our crop plants. The understanding of the mechanisms of stem cell proliferation in plants will provide a means to control the number, arrangement and size of organs produced during development, and can therefore be used to enhance crop plant productivity.

As can be seen from the foregoing, there is a continuing need in the art for identification of genes and proteins involved in stem cell proliferation, and organ development.

It is thus an object of the present invention to provide a novel gene and protein which regulates stem cell proliferation and development and which may be manipulated to improve health, productivity and yield of plants.

It is yet another object of the invention to provide a DNA sequence of a maize gene the product of which is involved in the regulation of inflorescence shoot meristem development.

A further object is to provide a mechanism for manipulating meristem proliferation and concomitant organ development to achieve increased yield, to control inflorescence number, arrangement or other reproductive development in plants.

A further object of the present invention is to provide genetic constructs for expression of or inhibition of this gene product, as well as antibodies for recognition of the same.

A further object of the invention is to provide a method for inducing fasciation in plant tissues.

Finally, it is an object of the present invention to provide genetic material which can used to screen other genomes to identify other genes with similar effects from other plant sources or even from animal sources.

Other objects of the invention will become apparent from the description of the invention which follows.

SUMMARY OF THE INVENTION

According to the invention a novel gene (*fae2*) has been isolated and characterized from maize. This gene encodes a receptor-like protein which is intimately involved in the regulation of inflorescence shoot meristem development. The *fae2* gene product is a member of the leucine rich repeat (LRR) class of transmembrane receptors. This group is characterized by a motif of around 24-29 amino acids often used for protein-protein interactions.

The gene encodes a protein product which is intimately involved in the regulation of meristem cell proliferation, particularly in inflorescence development. Mutants with disruptions in the (*fae2*) gene product demonstrate aberrant growth regulation during inflorescence formation resulting in fasciation.

Thus the novel gene and protein product of the invention provide a valuable tool for the manipulation of meristem growth, organ development, seed number, and flower arrangement, development and embryogenesis to increase yield, health and stability of plants. Genetic engineering methods known in the art can be used to inhibit expression of the gene or to further induce expression thus controlling the developmental effects regulated thereby, in not only maize but other plants and animals. Further, due to the conserved nature of these LRR receptor genes, it is expected that other such genes may be identified using the DNA and amino acid sequences herein to characterize other closely related genes from other species.

The invention further comprises novel compositions including protein products and nucleic acid sequences isolated from plants. Also included are expression constructs comprising these sequences as well as transformed cells, vectors and transgenic plants incorporating same. The invention further comprises monoclonal or polyclonal antibodies which recognize the novel proteins of the invention.

The invention also includes methods for manipulating organ development particularly flower development, seed development, meristem proliferation and yield of plants by incorporating the expression and or inhibition constructs of the invention.

Finally, the invention comprises a novel regulator, or promoter, region of this gene which has been isolated and characterized and can be used for temporal and spatial expression of heterologous gene products.

DESCRIPTION OF FIGURES

Figures 1A and 1B are diagrams depicting the structure and biology of the corn plant particularly the inflorescence structures.

Figure 2 is a photograph depicting the normal versus fasciated organs of corn plants including the ears, tassels and central spike.

Figure 3 is photograph showing Northern analysis of *fae2* expression. Total RNA, approximately 10μg/lane from normal ear primordia (5-15mm. *fae2* ear primordia, vegetative seedling apex, young, unexpanded leaf, expanded leaf and root tissues. Note the strong signal in normal ears, vegetative apex and young leaf tissue. Control probing with *knotted1* probe shows a signal in *fae2* ear lanes but not in leaf or root, as expected.

Figure 4 is a diagram depicting Sequence alignment of *fae2* and *CLV2, using* ClustalW version 1.8 (http://dot.imgen.bcm.tmc.edu:9331/multi-align/Help/clustalw.html);. *fae2* is on the top line and CLV2 is below. Identical residues are in black, similar are gray, dashes represent gaps introduced to optimize the alignment, and "empty" gaps are introduced to separate each LRR motif (Thomas et al., 1997). Arrows indicate the positions

of the Mu insertions in the two *fae2* alleles. Predictions of transmembrane and signal sequences are by SMART (http://smart.embl-heidelberg.de/) for *FAE2* and from (Jeong et al., 1999) for CLV2.

Figure 5 is a schematic of the *fae2* and CLV2 predicted motifs including signal sequence, LRR, transmembrane region and cytoplasmic tail.

Figure 6 is a depiction of the wild type fasciated ear 2 genomic sequence. The sequence includes 7235 base pairs including approximately 3736 base pairs of promoter and 5' UTR, and 1963 bp of 3' UTR and downstream sequence.

Figure 7 is the *fae2* promoter sequence and 5' UTR (3436 base pairs) ending with the ATG start codon of *fae2*.

Figure 8 is the *fae2* 3' untranslated region and downstream sequence of 1963 base pairs starting with the stop codon of *fae2*.

Figure 9 is the cDNA sequence (2323 bp) containing 43 base pairs upstream of the ATG ending in the polyA tail.

Figure 10 is the nucleotide sequence of the *fae2*-0 allele (3744 bp) nucleotide sequence containing 43 bp upstream of the ATG ending at the position of the PolyA tail, MU8 transposon sequence is in bold.

DETAILED DESCRIPTION OF THE INVENTION

Shoot development depends on the coordinated activities of groups of stem cells called meristems that are responsible for organ initiation and positioning. Within the meristem, a finely controlled balance between cell proliferation and incorporation of cells into new primordia ensures that the meristem is maintained as an organized structure. During inflorescence development the inflorescence apical meristem, which consists of a few hundred cells, supplies tens of thousands of cells to make spikelet primordia. In certain cases, which can be environmental or genetic in nature, the fine balance in the meristem is upset and it over proliferates, in a process known as fasciation.

In maize as in other plants there are several loci that mutate to give a fasciated ear phenotype, and this invention involves the discovery of a novel locus of this type, called fasciated ear2 (fae2). *fae2* encodes a leucine rich repeat protein which by analogy to similar proteins in other plants likely acts as a receptor to control meristem organization. *fae2* is expressed in inflorescence and floral meristems, and closer examination of *fae2* flowers reveals that their development and positioning is also abnormal. *fae2* shows a genetic interaction with ramosa3, a mutation that also causes fasciation in some genetic backgrounds, therefore it is likely that *fae2* and ra3 interact directly or closely in a pathway to control ear meristem development. Control of ear meristem size may be one mechanism by which the number of rows of spikelets is controlled, and it is possible that subtle changes in this class of genes was involved during the domestication of crop plants and the selection for example for larger fruits or increased numbers of seeds.

The *fae2* gene of maize encodes a receptor like protein that regulates the proliferation of stem cells in the maize inflorescences. Similarity of this gene to the *clv2* gene of *Arabidopsis* provides an indication that the clavata pathway might be conserved in maize. Maize is one of the most important crops worldwide, in part because of the tremendous morphological selection for a high yielding ear. A greater understanding of *fae2* and its role in inflorescence development will provide useful tools to improve productivity of maize and other cereal crops such as wheat or rice.

This invention involves the isolation and characterization of these novel proteins as well as their conservatively modified variants.

Definitions

Various terms relating to the compositions and methods of the present invention are used herein above and also throughout the specification and claims and unless otherwise indicated shall have the meaning specified herein.

Various units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in

amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range and include each integer within the defined range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. Unless otherwise provided for, software, electrical, and electronics terms as used herein are as defined in The New IEEE Standard Dictionary of Electrical and Electronics Terms (5th edition, 1993). The terms defined below are more fully defined by reference to the specification as a whole.

The term "antibody" includes reference to antigen binding forms of antibodies (e.g., Fab, $F(ab)_2$). The term "antibody" frequently refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). However, while various antibody fragments can be defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments such as single chain Fv, chimeric antibodies (i.e., comprising constant and variable regions from different species), humanized antibodies (i.e., comprising a complementarity determining region (CDR) from a non-human source) and heteroconjugate antibodies (e.g., bispecific antibodies).

An "antisense oligonucleotide" is a molecule of at least 6 contiguous nucleotides, preferably complementary to DNA (antigene) or RNA (antisense), which interferes with the process of transcription or translation of endogenous proteins so that gene products are inhibited.

A "cloning vector" is a DNA molecule such as a plasmid, cosmid, or bacterial phage that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites at which foreign DNA sequences can be

inserted in a determinable fashion without loss of essential biological function of the vector, as well as a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

A "coding sequence" or "coding region" refers to a nucleic acid molecule having sequence information necessary to produce a gene product, when the sequence is expressed.

The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also, by reference to the genetic code, describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; and UGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide of the present invention is implicit in each described polypeptide sequence and is within the scope of the present invention.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively

modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7, or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the native protein for its native substrate. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton (1984) Proteins W.H. Freeman and Company.

The term "co-suppression" is a method of inhibiting gene expression in plants wherein a construct is introduced to a plant. The construct has one or more copies of sequence which is identical to or which shares nucleotide homology with a resident gene.

As used herein the term "ear" shall not be limited to maize and shall include any developing female inflorescence from a plant.

By "encoding" or "encoded", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the

amino acid sequence is encoded by the nucleic acid using the "universal" genetic code. However, variants of the universal code, such as are present in some plant, animal, and fungal mitochondria, the bacterium *Mycoplasma capricolum*, or the ciliate *Macronucleus*, may be used when the nucleic acid is expressed therein.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray *et al.* *Nucl. Acids Res.* 17:477-498 (1989)). Thus, the maize preferred codon for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants are listed in Table 4 of Murray *et al.*, *supra*.

The term "expression" refers to biosynthesis of a gene product. Structural gene expression involves transcription of the structural gene into mRNA and then translation of the mRNA into one or more polypeptides.

An "expression vector" is a DNA molecule comprising a gene that is expressed in a host cell. Typically, gene expression is placed under the control of certain regulatory elements including promoters, tissue specific regulatory elements, and enhancers. Such a gene is said to be "operably linked to" the regulatory elements.

As used herein the term "*fae2*" shall include any of the fae2 amino acid sequences specified herein and their conservatively modified variants which retain the fae2 biological functions described herein. With respect to a "nucleotide sequence encoding fae2" the term includes nucleotide sequences which encode fae2 and its conservatively modified variants as well as those fae2 encoding nucleic acid sequences which hybridize under conditions of high stringency to the sequences disclosed herein.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

As used herein the term "high stringency" shall mean conditions or hybridization equivalent to the following: hybridized for 12 hours at 42°C in a buffer containing 50% formamide, 5 X SSPE, 2% SDS, 10 X Denhardt's solution, and 100 μg/ml salmon sperm DNA, and washing with 0.1 X SSC, 0.1% SDS at 55°C and exposed to Kodak X-Omat AR film for 4 days at -70°C.

"Homologous recombination" is another method of inhibiting gene function by introducing a disruption construct to a plant cell under conditions which facilitate recombination of endogenous genetic material with the construct.

By "host cell" is meant a cell which contains a vector and supports the replication and/or expression of the vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells. A particularly preferred monocotyledonous host cell is a maize host cell.

The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic

cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

With reference to nucleic acid molecules, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it was derived. For example, the "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a procaryote or eucaryote. An "isolated nucleic acid molecule" may also comprise a cDNA molecule.

With respect to RNA molecules, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form (the term "substantially pure" is defined below).

With respect to proteins or peptides, the term "isolated protein (or peptide)" or "isolated and purified protein (or peptide)" is sometimes used herein. This term may refer to a protein that has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form. Alternatively, this term may refer to a protein produced by expression of an isolated nucleic acid molecule.

As used herein the term "kernel" shall also not be limited to maize but shall include grain, or seed within a fruit.

As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

The term "nucleic acid construct" or "DNA construct" is sometimes used to refer to a coding sequence or sequences operably linked to appropriate regulatory sequences and inserted into a vector for transforming a cell. This term may be used interchangeably with the term "transforming DNA". Such a nucleic acid construct may contain a coding sequence for a gene product of interest, along with a selectable marker gene and/or a reporter gene.

By "nucleic acid library" is meant a collection of isolated DNA or RNA molecules which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, Vol. 152, Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.*, *Molecular Cloning – A Laboratory Manual*, 2nd ed., Vol. 1-3 (1989); and *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994).

The term "operably linked" or "operably inserted" means that the regulatory sequences necessary for expression of the coding sequence are placed in a nucleic acid molecule in the appropriate positions relative to the coding sequence so as to enable expression of the coding sequence. This same definition is sometimes applied to the arrangement other transcription control elements (e.g. enhancers) in an expression vector.

Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

As used herein, "polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide, or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as naturally occurring nucleotides and/or allow translation into the

same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons as "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including among other things, simple and complex cells.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, that protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring amino acids. The terms "polypeptide", "peptide" and "protein" are also inclusive of modifications including, but not limited to, phosphorylation, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation. It will be appreciated, as is well known and as noted above, that polypeptides are not entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular

polypeptides may be synthesized by non-translation natural process and by entirely synthetic methods, as well. Further, this invention contemplates the use of both the methionine-containing and the methionine-less amino terminal variants of the protein of the invention. With respect to a protein, the term "N-terminal region" shall include approximately 50 amino acids adjacent to the amino terminal end of a protein.

The terms "promoter", "promoter region" or "promoter sequence" refer generally to transcriptional regulatory regions of a gene, which may be found at the 5' or 3' side of the coding region, or within the coding region, or within introns. Typically, a promoter is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The typical 5' promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence is a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. The term promoter includes the essential regulatory features of said sequence and may optionally include a long terminal repeat region prior to the translation start site.

A "recombinant host" may be any prokaryotic or eukaryotic cell that contains either a cloning vector or an expression vector. This term also includes those prokaryotic or eukaryotic cells that have been genetically engineered to contain the clone genes in the chromosome or genome of the host cell.

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The term "reporter gene" refers to a gene that encodes a product which is easily detectable by standard methods, either directly or indirectly.

The term "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass non-natural analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

The term "selectable marker gene" refers to a gene encoding a product that, when expressed, confers a selectable phenotype such as antibiotic resistance on a transformed cell.

With respect to oligonucleotides or other single-stranded nucleic acid molecules, the term "specifically hybridizing" refers to the association between two single-stranded nucleic acid molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art i.e., conditions of stringency (sometimes termed "substantially complementary"). In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded DNA or RNA molecule, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence.

The term "stringent conditions" or "stringent hybridization conditions" includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and may be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which are 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected

(heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, optionally less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 50°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267-284 (1984): T_m=81.5°C + 16.6 (log M) + 0.41 (%GC) –0.61 (% form) – 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with ≥90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its

complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acids Probes*, Part I, Chapter 2, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995).

A "structural gene" is a DNA sequence that is transcribed into messenger RNA (mRNA) which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

The term "substantially equivalent" as used herein means that the peptide is a substance having an amino acid sequence with at least 30%-50% homology with at least one form of the protein as disclosed herein. 80% homology is preferred and 90% homology is most preferred especially including conservative substitutions. With respect to a nucleotide sequence the term substantially equivalent means that the sequence will encode a protein or peptide that is substantially equivalent.

Homology is calculated by standard methods which involve aligning two sequences to be compared so that maximum matching occurs, and calculating the percentage of matches. Substantially equivalent substances to these include those wherein one or more of the residues of the native sequence is deleted, substituted for, or inserted by a different amino acid or acids.

Preferred substitutions are those which are conservative, i.e., wherein a residue is replaced by another of the same general type. As is well understood, naturally occurring amino acids can be sub classified as acidic, basic, neutral and polar, or neutral and nonpolar. Furthermore, three of the encoded amino acids are aromatic. It is generally preferred that peptides differing from the native MEA sequence contain substitutions which are from the same group as that of the amino acid replaced. Thus, in general, the basic amino acids Lys and Arg are interchangeable; the acidic amino acids aspartic and glutamic are interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn are interchangeable; the nonpolar aliphatic acids Gly, Ala, Val, Ile, and Leu are conservative with respect to each other (but because of size, Gly and Ala are more closely related and Val, Ile and Leu are more closely related), and the aromatic amino acids Phe, Trp, and Tyr are interchangeable. While proline is a nonpolar neutral amino acid, it represents difficulties because of its effects on conformation, and substitutions by or for proline are not preferred, except when the same or similar conformational results can be obtained. Polar amino acids which represent conservative changes include Ser, Thr, Gln, Asn; and to a lesser extent, Met. In addition, although classified in different categories, Ala, Gly, and Ser seem to be interchangeable, and Cys additionally fits into this group, or may be classified with the polar neutral amino acids.

In general, whatever substitutions are made are such that the functional properties of the intact proteinaceous molecule is retained and ancillary properties, such as non-toxicity are not substantially disturbed.

A "transgenic plant" is a plant having one or more plant cells that contain an expression vector. Plant tissue includes differentiated and undifferentiated tissues or plants, including but not limited to roots, stems, shoots, leaves, pollen, seeds, tumor tissue, and various forms of cells and culture such as single cells, protoplasm, embryos, and callus tissue. The plant tissue may be in plant or in organ, tissue, or cell culture. These proteins can be used in techniques described herein as molecular markers in breeding to identify and/or select plants with improved heat and drought tolerance similar

to maize line ZPBL 1304, as these proteins were shown to be missing in drought and heat sensitive lines.

A "vector" is a replicon, such as plasmid, phage, cosmid, or virus to which another nucleic acid segment may be operably inserted so as to bring about the replication or expression of the segment.

Transmembrane receptors in plants

Transmembrane receptors play diverse roles in plant development and defense (Becraft, 1998; Lease et al., 1998; Torii, 2000). Many fall in the leucine rich repeat (LRR) class; the LRR is a motif of around 24-29 amino acids that is often used for protein-protein interactions (Kobe and Deisenhofer, 1994; Kobe and Deisenhofer, 1995). *Arabidopsis* contains many LRR receptor like kinases (He et al., 2000), but function has been determined for only a few; they are involved in organ growth, abscision, brassinosteroid perception, SAM development and plant defense (Torii et al., 1996; Jinn et al., 2000; Li and Chory, 1997; Clark et al., 1997; Song et al., 1995). Evidence that the LRRs are involved in ligand interaction has come from studying variation in disease resistance genes, reviewed in (Martin, 1999; Ellis et al., 2000; Richter and Ronald, 2000). Concordantly, the LRR and transmembrane/ juxtamembrane domains of the brassinosteriod receptor *BRI1* are sufficient for ligand perception (He et al., 2000). The extracellular domain of *BRI1* contains 25 LRRs flanked by pairs of cysteines, and an "island" of 70 amino acids between LRRs 21 and 22. Many mutations in *bri1* cluster in the island and the first LRR after the island, suggesting these parts of the predicted extracellular domain are important for ligand specificity (Li and Chory, 1997; Friedrichsen et al., 2000). However in *Cf4* and *Cf9*, LRR receptors for different fungal elicitors, the island regions are identical, and specificity is likely determined by the N terminal LRRs (Thomas et al., 1997). *Cf4* and *Cf9* encode transmembrane LRR proteins lacking a signaling domain, suggesting that they function as a dimer, perhaps with an LRR receptor like kinase (Jones et

al., 1994). Dimerization is a common feature for receptor tyrosine kinase activation in animal systems (Schlessinger, 2000).

The most well characterized LRR receptor-ligand system in plants is the *CLAVATA* system in *Arabidopsis*. *CLV1* encodes a transmembrane LRR-kinase, *CLV2* encodes a protein that like *Cf4* has predicted transmembrane and LRR domains with only a very short intracellular region, and *CLV3* encodes a small protein that is predicted to be secreted (Clark et al., 1997; Jeong et al., 1999; Fletcher et al., 1999). Mosaic analysis of *clv3* supports the notion that it encodes an intercellular signaling molecule (Fletcher et al., 1999). Whilst *CLV1* and *CLV3* expression are restricted to shoot meristems, *CLV2* is expressed more widely, consistent with its effect on other shoot organs. Biochemical studies have shown that CLV1 is present in two complexes; the smaller one consists of a disulfide linked multimer, whereas the larger complex was found to contain known components that include CLV3, the KAPP phosphatase, and a Rho related GTPase, both possible downstream effectors. CLV1 and CLV3 are required for formation of the large complex (Trotochaud et al., 1999; Trotochaud et al., 2000), and accumulation of CLV1 protein is severely reduced in *clv2* mutants (Jeong et al., 1999; Kayes and Clark, 1998). Together these results suggest that CLV1 and CLV2 proteins act as a heterodimer, perhaps disulfide linked through paired cysteine residues flanking the LRRs, and binding of CLV3 is required for assembly of a larger complex that includes downstream signaling effectors (Trotochaud et al., 1999; Trotochaud et al., 2000). Some of these predictions are supported by *clv3* overexpression studies (Brand et al., 2000). It should be noted that the lack of CLV2 antibodies means that the evidence for CLV2 in the complex is circumstantial, though the size of the disulfide linked fraction and the reduction of CLV1 protein in CLV2 mutants is fully consistent with the model.

Applicants have discovered a regulatory inflorescence meristem development gene isolated from maize that is involved in cell proliferation associated with seed development. The gene includes a signal sequence, a plurality of leucine rich repeat motifs interrupted by an island region, a

transmembrane domain and a cytoplasmic tail. Mutations in the *fae2* gene product as in Figure 9, causes the meristem to overproliferate resulting in phenotypic changes including increased inflorescence size and fasciation. Thus, the *fae* gene and protein product can regulate proliferation both negatively and positively depending upon the state of the gene. The wild type protein apparently acts to regulate meristem growth while the strongest mutant form promotes meristem over proliferation. A role in the control of meristem proliferation and development either positively or negatively has also been shown other fasciated mutants which demonstrate increase in yield (Luckwill 1943, Zulinski 1945), and it is contemplated that the sequences herein may be used to increase yield of recipient plants.

Thus in one embodiment of the invention, the fae2 gene or its protein product can be used in regulation of meristem proliferation, both if overexpressed or if the activity is suppressed by mutation or by other mechanisms such as for instance by antisense expression, homologous recombination or co-suppression mechanisms.

The invention herein in its broadest sense contemplates the discovery of the existence of an *fae2* gene in plants that is associated among other things with meristem proliferation in inflorescence development. The discovery of the existence of this type of gene creates numerous opportunities for manipulation of inflorescence development in plants in general. Due the highly conserved nature of the gene product, it is expected that this gene or ones substantially equivalent thereto may be identified from other plants with similar meristem specific functions. These homologs are intended to be within the scope of this invention. Similarly, the protein product disclosed here also many be used for other plants and many other mutants may be either engineered by those of skill in the art or isolated from other species. Homologous proteins or mutants as described herein and as isolated form other plants are also intended to be within the scope of this invention.

According to the invention, function of the *fae2* gene has been identified as controlling cell proliferation. Two mutant forms of the gene were identified

with insertional mutations which resulted in a fasciated phenotype in resulting plants.

This invention further contemplates methods of controlling organ development, cell proliferation, flower development etc by manipulating *fae2* genes in plants through genetic engineering techniques which are known and commonly used by those of skill in the art. Such methods include but are in no way limited to generation of increase seed number, flower or organ number, arrangement, size, etc., as well as other tissue specific regulation based upon expression of the gene at time, spatial and developmental periods.

In yet another embodiment the invention comprises regulatory sequences associated with the novel fae 2 gene of the invention. This regulatory region may be used to achieve expression of heterologous genes in tissues associated with and during periods of inflorescence development. The fae 2 gene product was shown to be expressed in the vegetative apex, inflorescences, and young leaf tissues. Expression is higher in less mature tissues.

The invention in one aspect comprises expression constructs comprising a DNA sequence which encodes upon expression a *fae2* gene product operably linked to a promoter to direct expression of the protein. These constructs are then introduced into plant cells using standard molecular biology techniques. The invention can be also be used for hybrid plant or seed production, once transgenic inbred parental lines have been established.

In another aspect the invention involves the inhibition of an *fae2* gene product in plants through introduction of a construct designed to inhibit the same gene product. The design and introduction of such constructs based upon known DNA sequences is known in the art and includes such technologies as antisense RNA or DNA, co-suppression or any other such mechanism. Several of these mechanisms are described and disclosed in United States Patent 5,686,649 to Chua et. al, which is hereby expressly incorporated herein by reference.

The methods of the invention described herein may be applicable to any species of plant.

Production of a genetically modified plant tissue either expressing or inhibiting expression of a structural gene combines the teachings of the present disclosure with a variety of techniques and expedients known in the art. In most instances, alternate expedients exist for each stage of the overall process. The choice of expedients depends on the variables such as the plasmid vector system chosen for the cloning and introduction of the recombinant DNA molecule, the plant species to be modified, the particular structural gene, promoter elements and upstream elements used. Persons skilled in the art are able to select and use appropriate alternatives to achieve functionality. Culture conditions for expressing desired structural genes and cultured cells are known in the art. Also as known in the art, a number of both monocotyledonous and dicotyledonous plant species are transformable and regenerable such that whole plants containing and expressing desired genes under regulatory control of the promoter molecules according to the invention may be obtained. As is known to those of skill in the art, expression in transformed plants may be tissue specific and/or specific to certain developmental stages. Truncated promoter selection and structural gene selection are other parameters which may be optimized to achieve desired plant expression or inhibition as is known to those of skill in the art and taught herein.

The following is a non-limiting general overview of Molecular biology techniques which may be used in performing the methods of the invention.

The nucleotide constructs of the present invention will share similar elements, which are well known in the art of plant molecular biology. For example, in each construct the DNA sequences of interest will preferably be operably linked (i.e., positioned to ensure the functioning of) to a promoter which allows the DNA to be transcribed (into an RNA transcript) and will comprise a vector which includes a replication system. In preferred

embodiments, the DNA sequence of interest will be of exogenous origin in an effort to prevent co-suppression of the endogenous genes.

PROMOTERS

The constructs, promoters or control systems used in the methods of the invention may include a tissue specific promoter, an inducible promoter or a constitutive promoter.

A large number of suitable promoter systems are available. For example one constitutive promoter useful for the invention is the cauliflower mosaic virus (CaMV) 35S. It has been shown to be highly active in many plant organs and during many stages of development when integrated into the genome of transgenic plants including tobacco and petunia, and has been shown to confer expression in protoplasts of both dicots and monocots.

Promoters (and other regulatory elements) may be heterologous (i.e., not naturally operably linked to a DNA sequence from the same organism). Promoters useful for expression in plants are known in the art and can be inducible, constitutive, tissue-specific, derived from eukaryotes, prokaryotes or viruses, or have various combinations of these characteristics.

In choosing a promoter to use in the methods of the invention, it may be desirable to use a tissue-specific or developmentally regulated promoter. A tissue-specific or developmentally regulated promoter is a DNA sequence which regulates the expression of a DNA sequence selectively in the cells/tissues of a plant critical to seed set and/or function and/or limits the expression of such a DNA sequence to the period of seed maturation in the plant. Any identifiable promoter may be used in the methods of the present invention which causes expression during stress as defined herein. It may also be advantageous to use an inducible promoter to provide expression of the construct during controlled periods.

These and other such promoters are known and accessible through sources such as Genbank.

Any inducible promoter can be used in the instant invention. *See* Ward *et al. Plant Mol. Biol.*22: 361-366 (1993). Exemplary inducible promoters

include, but are not limited to, that from the ACEl system which responds to copper (Mett *et al. PNAS* 90: 4567-4571 (1993)); In2 gene from maize which responds to benzenesulfonamide herbicide safeners (Hershey *et al., Mol. Gen. Genetics* 227: 229-237 (1991) and Gatz *et al., Mol. Gen. Genetics* 243: 32-38 (1994)) or Tet repressor from Tn10 (Gatz *et al., Mol. Gen. Genet.* 227: 229-237 (1991). A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone. Schena *et al., Proc. Natl. Acad. Sci. U.S.A.* 88: 0421 (1991).

Many different constitutive promoters can be utilized in the instant invention. Exemplary constitutive promoters include, but are not limited to, the promoters from plant viruses such as the 35S promoter from CaMV (Odell *et al., Nature* 313: 810-812 (1985) and the promoters from such genes as rice actin (McElroy et al., Plant Cell 2: 163-171 (1990)); ubiquitin (Christensen et al., Plant Mol. Biol 12: 619-632 (1989) and Christensen et al., Plant Mol. Biol. 18: 675-689 (1992)): pEMU (Last et al., Theor. Appl. Genet. 81: 581-588 (1991)); MAS (Velten et al., EMBO J. 3: 2723-2730 (1984)) and maize H3 histone (Lepetit et al., Mol. Gen. Genet. 231: 276-285 (1992) and Atanassova et al., Plant Journal 2 (3): 291-300 (1992)).

The preferred promoters may be used in conjunction with naturally occurring flanking coding or transcribed sequences of the fae2 genes or with any other coding or transcribed sequence that is critical to fae2 formation and/or function.

It may also be desirable to include some intron sequences in the promoter constructs since the inclusion of intron sequences in the coding region may result in enhanced expression and specificity. Thus, it may be advantageous to join the DNA sequences to be expressed to a promoter sequence that contains the first intron and exon sequences of a polypeptide

which is unique to cells/tissues of a plant critical to fae2 formation and/or function.

Additionally, regions of one promoter may be joined to regions from a different promoter in order to obtain the desired promoter activity resulting in a chimeric promoter. Synthetic promoters which regulate gene expression may also be used. The expression system may be further optimized by employing supplemental elements such as transcription terminators and/or enhancer elements.

OTHER REGULATORY ELEMENTS

In addition to a promoter sequence, an expression cassette or construct should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region or polyadenylation signal may be obtained from the same gene as the promoter sequence or may be obtained from different genes. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen et al., EMBO J. (1984) 3:835-846) or the nopaline synthase signal (Depicker et al., Mol. and Appl. Genet. (1982) 1:561-573).

Transport of protein produced by transgenes to a subcellular compartment such as the chloroplast, vacuole, peroxisome, glyoxysome, cell wall or mitochondrion, or for secretion into the apoplast, is accomplished by means of operably linking the nucleotide sequence encoding a signal sequence to the 5' and/or 3' region of a gene encoding the protein of interest. Targeting sequences at the 5' and/or 3' end of the structural gene may determine, during protein synthesis and processing, where the encoded protein is ultimately compartmentalized. The presence of a signal sequence directs a polypeptide to either an intracellular organelle or subcellular compartment or for secretion to the apoplast. Many signal sequences are known in the art. See, for example, Sullivan, T., "Analysis of Maize *Brittle*-1 Alleles and a Defective Suppressor-Mutator-Induced Mutable Allele", The Plant Cell, 3:1337-1348 (1991), Becker *et al., Plant Mol. Biol.*20: 49 (1992), Close, P.S., Master's Thesis, Iowa State

University (1993), Knox, C., *et al.*, "Structure and Organization of Two Divergent Alpha-Amylase Genes From Barley", *Plant Mol.Biol.* 9: 3-17 (1987), Lerner *et al., Plant Physiol.*91: 124-129 (1989), Fontes *et al.,Plant Cell* 3: 483-496 (1991), Matsuoka *et al., Proc. Natl. Acad. Sci.* 88: 834 (1991), Gould et al., *J. Cell Biol* 108: 1657 (1989), Creissen *et al., Plant J.* 2: 129 (1991), Kalderon, D., Robers, B., Richardson, W., and Smith A., "A short amino acid sequence able to specify nuclear location", *Cell* 39: 499-509 (1984), Stiefel, V., Ruiz-Avila, L., Raz R., Valles M., Gomez J., Pages M., Martinez-Izquierdo J., Ludevid M., Landale J., Nelson T., and Puigdomenech P., "Expression of a maize cell wall hydroxyproline-rich glycoprotein gene in early leaf and root vascular differentiation", *Plant Cell* 2: 785-793 (1990).

MARKER GENES

Recombinant DNA molecules containing any of the DNA sequences and promoters described herein may additionally contain selection marker genes which encode a selection gene product which confer on a plant cell resistance to a chemical agent or physiological stress, or confers a distinguishable phenotypic characteristic to the cells such that plant cells transformed with the recombinant DNA molecule may be easily selected using a selective agent. One such selection marker gene is neomycin phosphotransferase (NPT II) which confers resistance to kanamycin and the antibiotic G-418. Cells transformed with this selection marker gene may be selected for by assaying for the presence in vitro of phosphorylation of kanamycin using techniques described in the literature or by testing for the presence of the mRNA coding for the NPT II gene by Northern blot analysis in RNA from the tissue of the transformed plant. Polymerase chain reactions are also used to identify the presence of a transgene or expression using reverse transcriptase PCR amplification to monitor expression and PCR on genomic DNA. Other commonly used selection markers include the ampicillin resistance gene, the tetracycline resistance and the hygromycin resistance gene. Transformed plant cells thus selected can be induced to differentiate into plant structures

which will eventually yield whole plants. It is to be understood that a selection marker gene may also be native to a plant.

TRANSFORMATION

A recombinant DNA molecule whether designed to inhibit expression or to provide for expression containing any of the DNA sequences and/or promoters described herein may be integrated into the genome of a plant by first introducing a recombinant DNA molecule into a plant cell by any one of a variety of known methods. Preferably the recombinant DNA molecule(s) are inserted into a suitable vector and the vector is used to introduce the recombinant DNA molecule into a plant cell.

Selection of an appropriate vector is relatively simple, as the constraints are minimal. The minimal traits of the vector are that the desired nucleic acid sequence be introduced in a relatively intact state. Thus, any vector which will produce a plant carrying the introduced DNA sequence should be sufficient. Typically, an expression vector contains (1) prokaryotic DNA elements encoding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) DNA elements that control initiation of transcription, such as a promoter; (3) DNA elements that control the processing of transcripts such as transcription termination/polyadenylation sequences; and (4) a reporter gene. Useful reporter genes include β-glucuronidase, β-galactosidase, chloramphynical acetyltransferase, luciferase, kanamycin or the herbicide resistance genes PAT and BAR. Preferably, the reporter gene is kanamyacin or the herbicide resistance genes PAT and BAR. The BAR or PAT gene is used with the selecting agent Bialaphos, and is used as a preferred selection marker gene for plant transformation (Spencer, et al. (1990) J. Thero. Appl'd Genetics 79:625-631).

A general description of plant expression vectors and reporter genes can be found in Gruber, et al. (Gruber et al. (1993) Vectors for Plant

Transformation. In: Methods in Plant Molecular Biology and Biotechnology. Glich et al., eds. (CRC Press), pp. 89-119.

The use of Cauliflower Mosaic Virus (CaMV) (Howell, S.H., et al, 1980, Science, 208:1265) and gemini viruses (Goodman, R.M., 1981, J. Gen Virol. 54:9) as vectors has been suggested but by far the greatest reported successes have been with Agrobacteria sp. (Horsch, R.B., et al, 1985, Science 227:1229-1231).

Methods for the use of Agrobacterium based transformation systems have now been described for many different species. Generally strains of bacteria are used that harbor modified versions of the naturally occurring Ti plasmid such that DNA is transferred to the host plant without the subsequent formation of tumors. These methods involve the insertion within the borders of the Ti plasmid the DNA to be inserted into the plant genome linked to a selection marker gene to facilitate selection of transformed cells. Bacteria and plant tissues are cultured together to allow transfer of foreign DNA into plant cells then transformed plants are regenerated on selection media. Any number of different organs and tissues can serve as targets from Agrobacterium mediated transformation as described specifically for members of the Brassicaceae. These include thin cell layers (Charest, P.J., et al, 1988, Theor. Appl. Genet. 75:438-444), hypocotyls (DeBlock, M., et al, 1989, Plant Physiol. 91:694-701), leaf discs (Feldman, K.A., and Marks, M.D., 1986, Plant Sci. 47:63-69), stems (Fry J., et al, 1987, Plant Cell Repts. 6:321-325), cotyledons (Moloney M. M., et al, 1989, Plant Cell Repts. 8:238-242) and embryoids (Neuhaus, G., et al, 1987, Theor. Appl. Genet. 75:30-36). It is understood, however, that it may be desirable in some crops to choose a different tissue or method of transformation.

Other methods that have been employed for introducing recombinant molecules into plant cells involve mechanical means such as direct DNA uptake, liposomes, electroporation (Guerche, P. et al, 1987, Plant Science 52:111-116) and micro-injection (Neuhaus, G., et al, 1987, Theor. Appl. Genet. 75:30-36). The possibility of using microprojectiles and a gun or other device

to force small metal particles coated with DNA into cells has also received considerable attention (Klein, T.M. et al., 1987, Nature 327:70-73).

It is often desirable to have the DNA sequence in homozygous state which may require more than one transformation event to create a parental line, requiring transformation with a first and second recombinant DNA molecule both of which encode the same gene product. It is further contemplated in some of the embodiments of the process of the invention that a plant cell be transformed with a recombinant DNA molecule containing at least two DNA sequences or be transformed with more than one recombinant DNA molecule. The DNA sequences or recombinant DNA molecules in such embodiments may be physically linked, by being in the same vector, or physically separate on different vectors. A cell may be simultaneously transformed with more than one vector provided that each vector has a unique selection marker gene. Alternatively, a cell may be transformed with more than one vector sequentially allowing an intermediate regeneration step after transformation with the first vector. Further, it may be possible to perform a sexual cross between individual plants or plant lines containing different DNA sequences or recombinant DNA molecules preferably the DNA sequences or the recombinant molecules are linked or located on the same chromosome, and then selecting from the progeny of the cross, plants containing both DNA sequences or recombinant DNA molecules.

Expression of recombinant DNA molecules containing the DNA sequences and promoters described herein in transformed plant cells may be monitored using Northern blot techniques and/or Southern blot techniques known to those of skill in the art.

The regenerated plant are transferred to standard soil conditions and cultivated in a conventional manner.

After the expression or inhibition cassette is stably incorporated into regenerated transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

It may be useful to generate a number of individual transformed plants with any recombinant construct in order to recover plants free from any position effects. It may also be preferable to select plants that contain more than one copy of the introduced recombinant DNA molecule such that high levels of expression of the recombinant molecule are obtained.

As indicated above, it may be desirable to produce plant lines which are homozygous for a particular gene. In some species this is accomplished rather easily by the use of anther culture or isolated microspore culture. This is especially true for the oil seed crop *Brassica napus* (Keller and Armstrong, Z. flanzenzucht 80:100-108, 1978). By using these techniques, it is possible to produce a haploid line that carries the inserted gene and then to double the chromosome number either spontaneously or by the use of colchicine. This gives rise to a plant that is homozygous for the inserted gene, which can be easily assayed for if the inserted gene carries with it a suitable selection marker gene for detection of plants carrying that gene. Alternatively, plants may be self-fertilized, leading to the production of a mixture of seed that consists of, in the simplest case, three types, homozygous (25%), heterozygous (50%) and null (25%) for the inserted gene. Although it is relatively easy to score null plants from those that contain the gene, it is possible in practice to score the homozygous from heterozygous plants by southern blot analysis in which careful attention is paid to the loading of exactly equivalent amounts of DNA from the mixed population, and scoring heterozygotes by the intensity of the signal from a probe specific for the inserted gene. It is advisable to verify the results of the southern blot analysis by allowing each independent transformant to self-fertilize, since additional evidence for homozygosity can be obtained by the simple fact that if the plant was homozygous for the inserted gene, all of the subsequent plants from the selfed seed will contain the gene, while if the plant was heterozygous for the gene, the generation grown from the selfed seed will contain null plants. Therefore, with simple selfing one can easily select homozygous plant lines that can also be confirmed by southern blot analysis.

Creation of homozygous parental lines makes possible the production of hybrid plants and seeds which will contain a modified protein component. Transgenic homozygous parental lines are maintained with each parent containing either the first or second recombinant DNA sequence operably linked to a promoter. Also incorporated in this scheme are the advantages of growing a hybrid crop, including the combining of more valuable traits and hybrid vigor.

The following examples serve to better illustrate the invention described herein and are not intended to limit the invention in any way.

This invention further contemplates the identification of other polynucleotides encoding fae2 type proteins. Methods for identifying these other polynucleotides are known to those of skill in the art and will typically be based on screening for other cells which express *fae2*. Nucleotide sequences encoding this protein are easily ascertainable to those of skill in the art through Genbank or the use of plant protein codon optimization techniques known to those of skill in the art and disclosed in the references disclosed herein (for example see EPO publication number 0682115A1 and Murray et al., 1989, Nuc Acid Res., Vol. 17 No. 2, pp 447-498, "Codon Usage in Plant Genes". It is preferred to use the optimized coding sequences, for the plant recipient species. These sequences can be used not only in transgenic protocols but as tags for marker-assisted selection in plant breeding programs.

The present invention also provides antibodies capable of binding to fae2 from one or more selected species. Polyclonal or monoclonal antibodies directed toward part or all of a selected fae2 gene product may be prepared according to standard methods. Monoclonal antibodies may be prepared according to general methods of Köhler and Milstein, following standard protocols

Purified fae2, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies which may serve as sensitive detection reagents for the presence and accumulation of the proteins in cultured cells or tissues and in intact organisms. Recombinant techniques enable expression of fusion

proteins containing part or all of a selected fae2. The full length protein or fragments of the protein may be used to advantage to generate an array of monoclonal or polyclonal antibodies specific for various epitopes of the protein, thereby providing even greater sensitivity for detection of the protein.

Polyclonal or monoclonal antibodies immunologically specific for fae2 may be used in a variety of assays designed to detect and quantitate the proteins. Such assays include, but are not limited to, (1) immunoprecipitation followed by protein quantification; (2) immunoblot analysis (e.g., dot blot, Western blot) (3) radioimmune assays, (4) nephelometry, turbidometric or immunochromatographic (lateral flow) assays, and (5) enzyme-coupled assays, including ELISA and a variety of qualitative rapid tests (e.g., dip-stick and similar tests).

Polyclonal or monoclonal antibodies that immunospecifically interact with fae2 can be utilized for identifying and purifying such proteins. For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules.

The following examples and intended to further illustrate the invention and are not intended to limit the invention in any way. The examples and discussion herein may specifically reference maize, however the teachings herein are equally applicable to any other cereal, grain, or flowering crop.

EXAMPLE 1

Phenotypic analysis of fae2 mutants.

Maize inflorescence structure.

Maize has two distinct inflorescence structures, the terminal tassel is branched and develops male flowers, whereas the ears bear female flowers, are unbranched, and develop on axillary shoots. Despite these differences, the tassel and ear start out as remarkably similar structures (Cheng et al., 1983).

The inflorescence meristem (IM) is derived from a vegetative SAM, and initiates a number of branch or spikelet pair meristems (BM or SPM) on its flanks. The fate of these meristems is variable, they can form long branches, such as those formed at the base of the tassel, or short branches consisting a pair of spikelet meristems (SMs). SMs in turn form a pair of floral meristems from which the florets develop (reviewed in (McSteen et al., 2000)).

The decision to develop into a long branch or a short branch (spikelet pair) is controlled in part by meristem identity genes such as *ramosa1, 2 and 3* (Neuffer et al., 1997; Postlethwait and Nelson, 1964).

The ear forms many rows of spikelet pair meristems in a polystichous phyllotaxy. Ultimately, the number of seed rows is determined by the number of SPMs initiated by the IM. The cob structure of the maize ear is rather unique among the grasses (Kellogg and Birchler, 1993), and is evolutionarily derived from the distichous inflorescence of teosinte (Beadle, 1980; Doebley, 1992). Whereas teosinte inflorescences have two rows of single spikelets, primitive maize lines have four rows of paired spikelets, so from teosinte to maize seed row number increased from two to eight. Modern maize lines have 8 to 18, and some varieties have up to 36 rows (USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network; http://www.ars-grin.gov/cgi-bin/npgs/html/obvalue.pl?89029). Row number in modern maize inbreds is controlled predominantly by genetic rather than environmental factors (Emerson and Smith, 1950). Clearly, increase in row number was a major yield factor in the development of maize as a crop, and row number or spikelet density variants are of importance in other cereals such as barley, wheat and rice (Futsuhara et al., 1979). In addition to classical mutant studies, geneticists and breeders have used QTL analysis to identify loci involved in inflorescence development, as described in the following section.

fae2 ears are severely fasciated, and usually shorter than normal (Fig . 2). In the B73 inbred, the number or rows of kernels varies from 26-36, compared to 16-18 for normal sibs. *fae2* tassels have a less severe phenotype,

they are not fasciated (i.e. not flattened or abnormally branched), but the central spike is thicker and the density of spikelets is higher (Fig. 2); normal sib tassels had a mean spikelet density of 4.5+/- 0.3 spikelets/ cm, whereas *fae2-0* tassels (introgressed 4X into B73) had a density of 5.3 +/- 0.6 (n= 8-12 tassels). We have also found that *fae2* mutants are defective in spikelet pair meristem and floral development (Fig. 3).

2. *fae2* cloning and expression.

According to the invention *fae2* was isolated by making a genomic library and cloning a 16kb BamH1 fragment containing a *Mu8* co-segregating band. The Mu8 element disrupted a single predicted ORF of 613 amino acids. To prove this was the correct gene we isolated a new allele of *fae2,* by directed *Mu* tagging. The original allele, *fae2-0,* contains the *Mu8* transposon 412 nucleotides after the ATG (Figure 9). The second allele, *fae2-846,* has a *Mu7* element at nucleotide 714 after the ATG (Fig 5). *fae2-846* homozygotes have a fasciated ear phenotype that is indistinguishable from *fae2-0* (not shown). We isolated the *fae2* cDNA using 3' and 5' RACE PCR. Alignment with genomic sequence from the *Mu* flanking clone indicates the *fae2* coding sequence has no introns. SMART (http://smart.embl-heidelberg.de/) analysis indicates *fae2* is member of the leucine rich repeat (LRR) family of receptor like proteins. Specifically, *FAE2* is predicted to contain a signal sequence, LRRs interrupted by a 40 amino acid spacer or island region, a transmembrane domain and a 15 amino acid cytoplasmic tail. A BlastP (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) search indicated that the most similar sequence to *fae2* is CLV2 from *Arabidopsis* (score 376 , E value e^{-103}, approx 44% amino acid identity over most of the sequence, 58% similarity, see Fig. 4 for alignment). *fae2* has 4 less LRRs than CLV2 (Fig. 4).

We have sequenced 5kb of upstream region and 2kb of 3' region from the genomic *Mu* flanking clone. The 3' sequences and 3.5kb of upstream sequence do not pick up any significant match by Blast searches, sequence further upstream are related to retrotransposons. Northern analysis indicates that *fae2* is expressed in vegetative apex, inflorescence, and leaf tissues, with

expression being higher in less mature tissues (Fig. 4). *fae2* message is not detected in RNA extracted from ears of *fae2-0* mutant plants, consistent with the presence of a *Mu* element in the coding sequence. Because of this, we suspect that *fae2-0* is a null mutation, this is supported by a genetic analysis of hemizygous *fae2-0* plants, created using a TB 4L translocation stock, which have a similar phenotype to *fae2-0* homozygotes, so *fae2-0* behaves genetically as a null (Muller, 1932).

EXAMPLE 2

fae2 maps to a quantitative trait locus for row number.

fae2 was mapped using recombinant inbreds (Burr et al., 1988) to 4L near the centromere, and maps to a QTL for row number, a measure of the number of vertical rows of seeds on the maize ear. This is one of four significant QTL for row number mapped from $F_{2:3}$ progeny families of a cross between Mo17 and H99 inbred lines.

Null alleles of *fae2* have a massive over proliferation of the inflorescence meristem (IM), and up to double the number of rows of seed on the ear. A weak *fae2* allele could lead to a less dramatic increase in IM size and a modest increase in row number. This is based on an observed correlation between meristem size and phyllotaxy- in short a bigger meristem can initiate more organs simultaneously (Wardlaw, 1949; Green, 1987; Jackson and Hake, 1999). This model depends on the testable hypothesis that inflorescence meristem (IM) size correlates with row number, IM size was measured in the scanning EM for two maize inbred lines that differ in row number. IM diameter was measured from 12 immature ear primordia that were 8-15mm long, when the IM is still initiating spikelet pair meristems. B73 has more rows than Mo17, and we found it has a significantly larger IM (Fig. 6). The large standard deviation is expected due to the dynamic changes in meristem size at different stages of organ initiation (Jackson and Hake, 1999). Therefore IM size correlates positively with row number in the two lines.

This is supported by the tassel phenotype of *fae2*. No abnormal flattening or splitting (fasciation) in *fae2* tassels was observed, rather a moderate increase in the thickness of the central spike (Fig. 2) and an increase in spikelet density. Spikelet density in the tassel can be thought of as equivalent to row number on the ear; the two traits appear different because of the different phyllotaxy and floral structures in the tassel compared to the ear. However both traits are dependent on the number of spikelet pair meristems initiated by the inflorescence meristem. Therefore the tassel phenotype can be perhaps considered a "weak" *fae2* phenotype, and the result is an increase in spikelet density without fasciation.

EXAMPLE 3

The link between *fae2*, IM size and a row number QTL.

According to the invention allelic variation in *fae2* will alter inflorescence meristem (IM) size, with a resulting effect on kernel row number. This is based on the observations that *fae2* maps to a QTL for row number, and that IM size correlates with row number differences between B73 and Mo17.

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What is claimed is:

1. A purified and isolated nucleotide sequence which encodes upon expression an *fae2* protein

2. The nucleotide sequence of claim 1 wherein said sequence comprises SEQ ID NO:1.

3. An expression construct comprising: a nucleotide sequence according to claim 1, operatively linked to a regulatory region capable of directing expression of a protein in a plant cell.

4. A vector capable or transforming or transfecting a host cell, said vector comprising an expression construct according to claim 3.

5. The vector of claim 4 wherein said vector is a plasmid based vector.

6. The vector of claim 4 wherein said vector is a viral based vector.

7. A prokaryotic or eucaryotic host cell transformed or transfected with a vector according to claim 4.

8. The host cell of claim 7 wherein said cell is a plant cell.

9. A fae2 protein which exhibits the following characteristics: a signal sequence, a plurality of LRRs, a transmembrane domain and cytoplasmic tail, wherein said protein functions to modulate meristem development.

10. The protein of claim 9 wherein said protein comprises an amino acid sequence according to SEQ ID NO:2.

11. The protein of claim 9 wherein said protein is expressed in a plant cell.

12. A method for controlling plant meristem proliferation comprising: introducing to a plant cell a genetic construct comprising a nucleotide sequence which encodes an *fae2* mutant protein so that meristem development is over stimulated; and said nucleotide sequence being operably linked to promoter and regulatory regions capable of inducing expression in a plant cell.

13. The method of claim 12 wherein said cell proliferation is increased.

14. The method of claim 13 wherein said increased cell proliferation comprises meristem cells.

15. The method of claim 12 wherein cell proliferation causes increased row number in maize.

16. A method of inducing cell over proliferation of meristem in plants comprising: inhibiting the expression of nucleotide sequence which encodes upon expression an *fae2* protein.

17. The method of claim 16 wherein said inhibition is by antisense.

18. The method of claim 17 wherein inhibition is by co-suppression.

19. The method of claim 16 wherein said inhibition is by homologous recombination.

20. A transmembrane protein receptor protein capable of increasing meristem cell proliferation said sequence comprising: the amino acid sequence of SEQ ID NO:2 including its conservatively modified variants.

21. A method of regulating meristem development in plants comprising: introducing to a plant cell a genetic construct, said construct being which will express or inhibit the *fae2* regulatory gene.

22. The method of claim 21 wherein said construct is an expression construct.

23. The method of claim 22 wherein said construct is an inhibition construct.

24. A method of identifying genes in plant species which regulate meristem development comprising: screening the genome of said plant species for a sequence that is homologous to SEQ ID NO:1 or a region of at least 100 bases thereof.

25. An antibody which is immunologically specific for one or more epitopes of *fae2* protein.

26. The antibody of claim 25 wherein said antibody is polyclonal.

27. The antibody of claim 25 wherein said antibody is monoclonal.

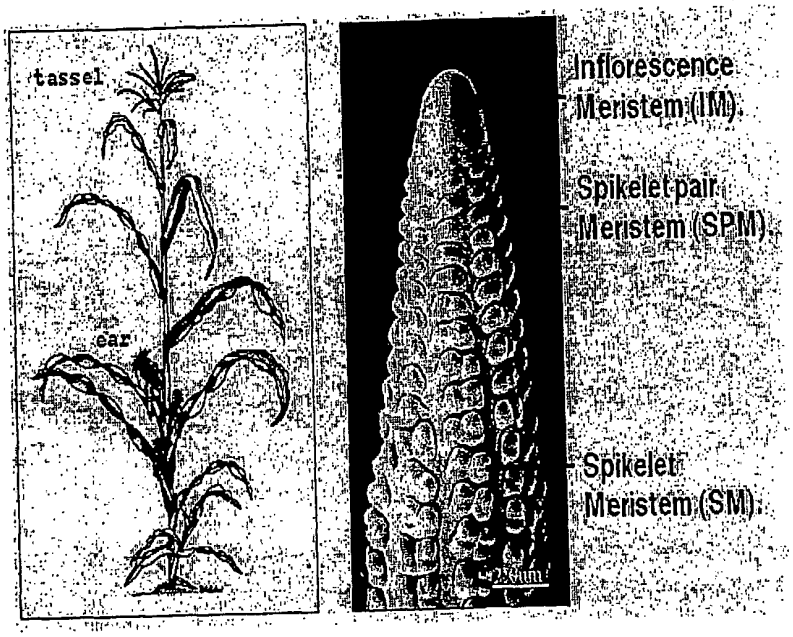

tassel
ear
Inflorescence Meristem (IM)
Spikelet pair Meristem (SPM)
Spikelet Meristem (SM)

FIGURE 1

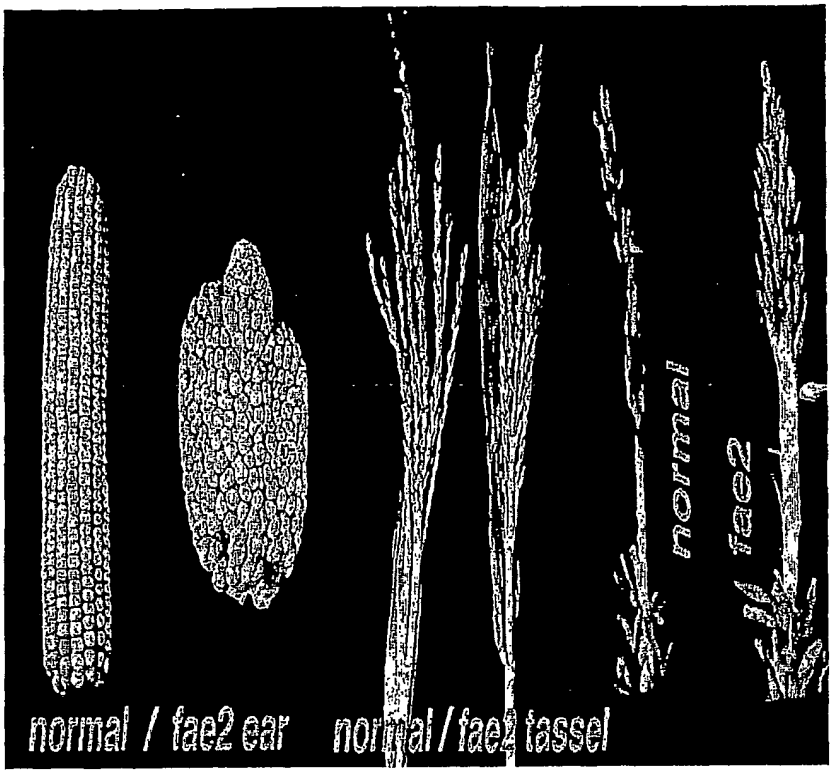


FIGURE 2

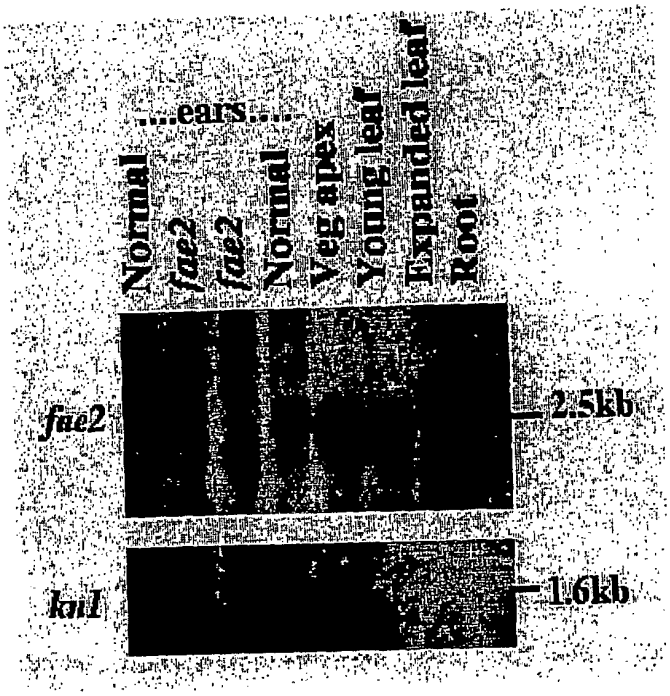
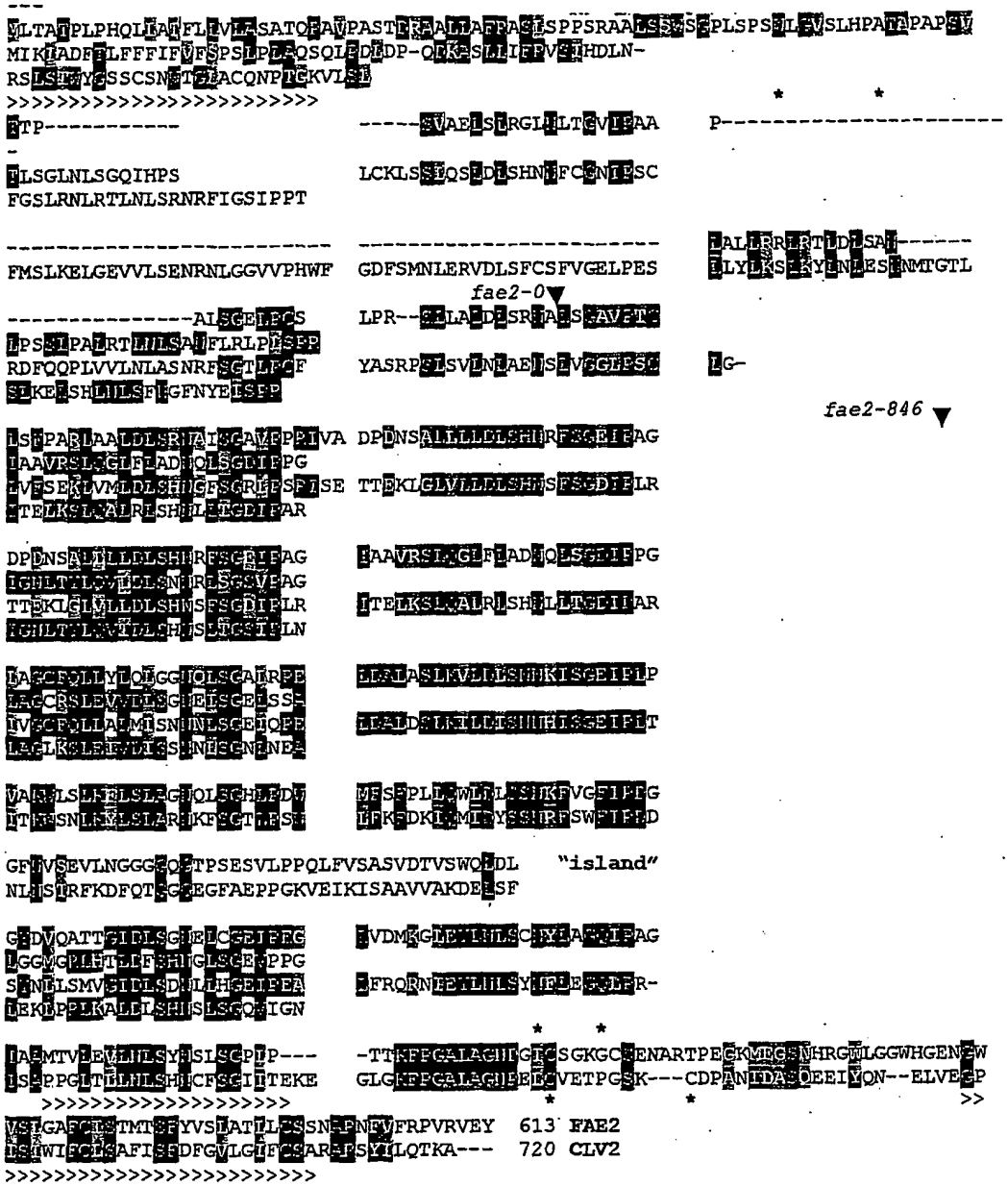
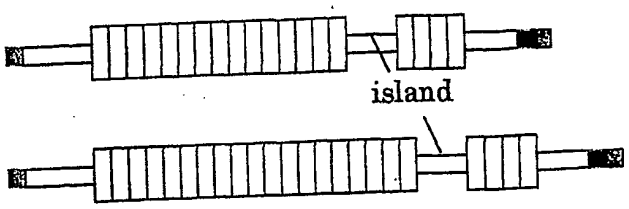

....ears....
Normal
fae2
fae2
Normal
Veg apex
Young leaf
Expanded leaf
Root
fae2
2.5kb
ku1
1.6kb

FIGURE 3

Fig 4. ClustalW alignment of FAE2 and CLV2.





FAE2

CLV2

Signal sequence LRR Transmembrane region Cytoplasmic tail

***Fasciated ear 2* genomic sequence; 7235bp, 11/15/00**

Includes approx 3736bp of promoter and 5' UTR, and 1963bp of 3' UTR and downstream sequence

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>*fae2* promoter and 5'UTR 3436bp 11/15/00
this sequence ends with the "ATG" start codon of *fae2*.

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ACCACCAGTCCACCACC**ATG**

>*fae2* 3' UTR and downstream sequence 1963 bp, 11/15/00
this sequence starts with stop codon of *fae2* (bold).

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AATGATCAACAAATGTATTTATTGCAAAGTGTGAGACAAGGCAACATACATATCTC
TGTTTTGTGCATTACCAAACTAACCCAGGCTGTAATTGCAGATTGATAATTCCTAT
AGCCGTAGCTTCTTCAGCTGGATAAGGTGGAGAATAAGCATCAGTGGCTTTTCAAA
GGGTTCGGCACTTGTGCAGAATCAGACTCATGCAGGAATCCGGTTCTAACTTTTCC
ACTAAAACCTTTTTCCTTTCTGAACTTGCTGCAGTACCTACCACTACTTGTAATGT
TGGATCAGTTTCACAGTTAACCACAGAATTTCATTGCTTTTAATCAAACGTGAAAT
GGTCACACAGAAAAACCAACATCCTTCAGAACCGCTCGTTTTATCTTGTTCTTTAG
ATTACTTCAAATGTGAGCGCTTTGAGATGGTCACCTAAGGTGTGTTTGGTTGATGT
ACTAAGGTGTGTTTGGTTGGATGTACAAAGAGTGATGGAAGGGGGCAACAACAGTT
TCTATAGTGTTTGGATGAGTCACGTGAGGACGAGATAAACATTAGAGTAATTTTTT
ATGGCGGCGTGATCCCAAAAAATCGAGGGAACGGTGTTATTCCTGCCTTATCCTAT
TTTGATCTCAAACCAAACACATCCTAAACTGTCTTGGATCTAGATAAGGTGACCTG
GACGCAAACGACGACTGCTGATGGTTGGTCTCCTGTCAGGAACTGGCTCGTCTGGC
CTAGACAAAAGAACTGGCTCGTCTGGCCTAGACAAAACTTTCATGTGGCCGTTGGC
CGTTGACGTTAAGAGGAATTGAGATCACCGCCGGCGCCAGGTCTATCATCTGATCT
GCATGCTCATCCTCATCACGCGAGTCCATTTCAGTTGTGCTCCCGATTTTTTGCCC
TGTGATTGCCATTGATTCGGCCTTCTGCATATGCTCGCATACGGCATTACAAAACA
CTCCACACAAATCTTGAAGTGCAAGCTATAGACGAAAAATCACTAAGACATTATAA
TTCTACCATTGAAAAATCACTAAGACATTACAATTCTACCATTAAACCGCCATCCA
TGTTTTACAAAAATCTCCATAGCAACCATCTCTAAAGAGTGATAAACATCGTTTAT
TTCCTGATGAGTTTTTTCTTTCTGCATCAGTCTCAAATTTGAACGAGCAGGTTCTC
CTAAAAATGACATGCATAATGGACAATATTTATTTATTGTTAAAAACAACATTTTA
TTT

***fae2* cDNA sequence (2323bp)Nucleotide sequence, containing 43 bp upstream of the ATG, ending in the poly A tail.**

AGGTTCTGCCGGCCACCGCCACCACCACCACCAGTCCACCACCATGCTGA
CAGCCACTCCCCTACCCCATCAGCTCCTGGCCACCTTCCTCCTCGCCCTG
GCGTCGGCGACCCAACCTGCAGTCCCTGCCTCCACCGACCGCGCAGCGCT
TCTCGCCTTCCGCGCGTCCCTGTCGCCGCCCTCCCGCGCCGCTCTATCCT
CGTGGAGCGGCCCGCTCTCGCCATCCTGGCTCGGCGTGTCGCTCCACCCC
GCCACGGCGCCAGCCCCTTCGGTCACCACTCCCTCCGTTGCCGAACTCTC
GCTCCGGGGCCTCAACCTCACGGGCGTGATCCCCGCGGCGCCGCTCGCGC
TCCTCCGACGTCTCCGGACGCTCGACCTCTCCGCCAACGCGCTTTCGGGA
GAGCTTCCCTGCTCCCTCCCGCGCTCGCTCCTCGCGCTCGACCTCTCCCG
CAACGCGCTCTCGGGGGCTGTCCCCACCTGCCTGCCGTCCTCGCTCCCCG
CGCTCCGCACCCTCAACCTCTCCGCCAACTTCCTCCGCCTCCCGCTCTCC
CCGCGTCTCTCCTTCCCCGCGCGCCTCGCTGCCCTTGATCTCTCCCGCAA
CGCCATCTCCGGCGCCGTCCCGCCGCGGATCGTCGCCGACCCCGACAACT
CCGCTCTCCTCCTCCTCGACCTCTCCCACAACCGCTTCTCCGGCGAGATC
CCCGCCAGTATCGCAGCCGTACGGAGCCTGCAGGGGCTTTTTCTCGCGGA
CAACCAGCTTTCCGGGGACATTCCTCCGGGGATAGGGAACCTGACCTATT
TGCAGGTGCTGGATTTGTCGAATAACCGATTGTCCGGTTCAGTGCCTGCC
GGACTTGCAGGCTGCTTCCAGCTTCTGTACCTGCAGCTTGGGGGTAACCA
GCTCTCTGGGGCACTCCGTCCGGAGCTCGACGCACTAGCTAGTCTCAAGG
TTCTAGATTTGTCGAATAACAAGATATCTGGGGAGATTCCCCTGCCGCTG
GCTGGGTGCAGGTCTTTGGAGGTGGTGGACTTGTCAGGAAATGAGATCTC
CGGTGAGCTCAGCAGTGCTGTAGCGAAATGGCTGAGCTTGAAGTTCTTAT
CACTGGCTGGTAACCAGCTCTCCGGCCACCCACCTGACTGGATGTTCTCG
TTCCCCCTGCTCCAGTGGCTTGATTTGTCTAGTAATAAGTTTGTGGGTTT
CATCCCAGATGGGGGGTTCAATGTCAGTGAAGTGCTTAACGGTGGAGGTG
GTCAGGGGACTCCATCAGAGAGTGTGCTTCCACCCCAATTGTTTGTGTCA
GCTTCTGTGGACACGGTGTCATGGCAGTTGGATTTGGGGTATGATGTTCA
GGCAACTACTGGTATAGACCTGTCTGGGAATGAGCTCTGTGGGGAGATAC
CAGAAGGGTTGGTTGACATGAAGGGGTTGGAGTATTTGAACCTCTCCTGT
AATTACTTGGCTGGGCAGATCCCTGCGGGGCTTGGGGGCATGGGGAGGTT
GCATACGCTTGACTTCTCACATAATAGGCTGTCAGGGGAGGTGCCTCCTG
GAATTGCAGCCATGACAGTGCTTGAGGTGCTTAACCTCTCCTACAATAGC
CTGTATGGGCCTTTGCCAACAACGAAGTTCCCAGGAGCATTAGCTGGAAA
CCCAGGAATTTGCAGTGGGAAAGGGTGCTCTGAGAATGCAAGGACTCCAG
AAGGGAAAATGGAAGGTAGCAATCACCGCGGTTGGCTTGGTGGCTGGCAT

GGAGAGAATGGATGGGTATCTCTTGGTGCATTTTGTATCAGCACAATGAC
TAGCTTCTATGTATCATTAGCAACCTTACTATGCTCCTCTAATGCAAGAA
ACTTCGTGTTTCGGCCTGTGAGGGTTGAATATTAACAAGAGGGGAAATTG
CAAAATCAGGTTGTTTTGAAGTTCGAGCGACTCTGGTCTGCAGCTGATTA
ACAAGAAATATGAGCATATGAGATGGATATCTTCAGCCAAGAGGAAGTGC
TGTCTCTTTTAATGATCAATCAAGCTCTCTTGATTGTTTCCTAATATTCT
TGATCTTGGGATGTGTAGATCTAGTTCTAATATTCCTACTGTTATAGAAT
GCAATCACCTGCTGGTGCTTGGTTGTAGCCCTGGCGTGTTTGGAGGATTG
GACACCAAGGATGCACATAATTTGAAGCGCTGGTACTGTGAACCACTTCA
GATGTAAATATTTTCTTTGGTTTTTAGTTCTGATCTAGTTTAAAACTGGA
CATGTATTTAGTGTTGTTGAGCTACCTTTCGATGTTATATTATGTCAATT
TGCTGGAAAAAAAAAAAAAAAA

FAE2 amino acid sequence 613 amino acids.

MLTATPLPHQLLATFLLALASATQPAVPASTDRAALLAFRASLSPPSRAA
LSSWSGPLSPSWLGVSLHPATAPAPSVTTPSVAELSLRGLNLTGVIPAAP
LALLRRLRTLDLSANALSGELPCSLPRSLLALDLSRNALSGAVPTCLPSS
LPALRTLNLSANFLRLPLSPRLSFPARLAALDLSRNAISGAVPPRIVADP
DNSALLLLDLSHNRFSGEIPASIAAVRSLQGLFLADNQLSGDIPPGIGNL
TYLQVLDLSNNRLSGSVPAGLAGCFQLLYLQLGGNQLSGALRPELDALAS
LKVLDLSNNKISGEIPLPLAGCRSLEVVDLSGNEISGELSSAVAKWLSLK
FLSLAGNQLSGHPPDWMFSFPLLQWLDLSSNKFVGFIPDGGFNVSEVLNG
GGGQGTPSESVLPPQLFVSASVDTVSWQLDLGYDVQATTGIDLSGNELCG
EIPEGLVDMKGLEYLNLSCNYLAGQIPAGLGGMGRLHTLDFSHNRLSGEV
PPGIAAMTVLEVLNLSYNSLYGPLPTTKFPGALAGNPGICSGKGCSENAR
TPEGKMEGSNHRGWLGGWHGENGWVSLGAFCISTMTSFYVSLATLLCSSN
ARNFVFRPVRVEY*

fae2-0 allele sequence 3744bp

Nucleotide sequence, containing 43 bp upstream of the ATG, ending at the position of the poly A tail, MU8 TRANSPOSON SEQUENCE IS IN BOLD.

AGGTTCTGCCGGCCACCGCCACCACCACCACCAGTCCACCACCATGCTGACAGCCACTC
CCCTACCCCATCAGCTCCTGGCCACCTTCCTCCTCGCCCTGGCGTCGGCGACCCAACCT
GCAGTCCCTGCCTCCACCGACCGCGCAGCGCTTCTCGCCTTCCGCGCGTCCCTGTCGCC
GCCCTCCCGCGCCGCTCTATCCTCGTGGAGCGGCCCGCTCTCGCCATCCTGGCTCGGCG
TGTCGCTCCACCCCGCCACGGCGCCAGCCCCTTCGGTCACCACTCCCTCCGTTGCCGAA
CTCTCGCTCCGGGGCCTCAACCTCACGGGCGTGATCCCCGCGGCGCCGCTCGCGCTCCT
CCGACGTCTCCGGACGCTCGACCTCTCCGCCAACGCGCTTTCGGGAGAGCTTCCCTGCT
CCCTCCCGCGCTCGCTCCTCGCGCTCGACCTCTCCCGCAACGCGCTCTCGGGGGAGATA
ATTGTCATTATAGACGAAGAGCGGACGGGATTCGACGAAATGGAGGCGATGGCGTTGGC
TTCTCTGTTCTGGAAACGCAGACGACAGCCAAACGCCAAAACGGAAAGGAGACAGCGCT
TGGAGCTGTGTAAACAGGTATTAGTCTCCTGTCCCCGTTTACCGTTCGCCTGCGCAGAC
GCCGTCTGGCATACTCCTCTCTACGCCGTCTCTTCTTGCGGCTGCTCTCGGGGTCGGCC
TGCTCGCAATACCTGTTTTGACACAAGCAGCGCCGGAGCGGGCCGCGCAGGTTGGCCTC
GAACAGCCCCGTGATCTTGCGCTCGCGTTGAACGCGCCACGGTGCGCAGGTTGGCCACG
GCTCGCCCGCGATCTGCGTGGCCCTGGCGTGCGCGGCCTCCAGGTCCCCCGAGAAGCCC
TTCATGAACATCTTCTGCAGCACGGTGGCGCCCACGACGAGCGGGAACACGGCGAGGAG
CACGAGCGCGAGGCGCACTGGAGGACGAACCCCGCGGTGTCAGGCCACCAGCATCAGCG
CCGAGTTCTGGACGATGACGGAGATGCGGTCCCCGATGGCGGACGGCACGTTCTGGGCG
TCCAGCGCGAGCCTGGCGCCACGCGCGCGCTGGCGTTCTCGTCCGTGTCGAACCAGGCG
ATCTCGTTGCGGAGCACGGCGTCGAACATCTTCTCGCGCACCCGCTTGGTCAGGTTCTC
GCCCACCGTGTCCCAGAACACGTGCTGCACCGTGTTGAACAGCAGCGCGCGAGGACATG
CCGATGAGCAGGTAGCAGTATTTGGCGATCTCGCGCTTCATGTACCGCGGGTCCGGCGC
GTAGTACACGCTGAGCACGGCGCTGAGGATGTAGGCGAAGATGGCGCTGAAGGAGCCGC
GGACCATGGAGCCGGCGAGCGCGTAGGCCCACTCGGGCGAGTTCATCCTGGCGAGGCGC
AGGAAGGAGCTGGCGCCGGCGCGGAACGCCAGCTGCTTGTCGGCCATGGTCCGGTGGTG
GTGGTGCGGGTCGTGGATGGAGAGGGTGAAGTCGGAGGTGGAGAAGTCGGAGAGGCGGC
GGGAGTAGGGGGAGCGGCCGTAGAGGAGTTGCGCGTCATGATGGGCGAGCTGACGGAGT
TGCGGGCGCTGGAGGGCCTGGCGCTGCGCCGTGCTGGTGCAATGTCGACCCCGAGAGCA
TGAACACGAGAGCATGAACACGAAACGGCGGCTAGGGCAGCGTCTGCGCAGACGAACGG
TAAACGGGGACAGGAGACTAATACCTGTTTACGCAGCTCCAAGCGCTGTCTTCTTTCCG
TTTTGGCGTTTGGCTGTCGCCTGCGTCTCCAGAACAGAGAAGCCAACGCCATCGCCTCC
ATTTCGTCGAATCCCGTCCGCTCTTCGTCTATAATGGCAATTATCTCCGCTCTCGGGGG
CTGTCCCCACCTGCCTGCCGTCCTCGCTCCCCGCGCTCCGCACCCTCAACCTCTCCGCC
AACTTCCTCCGCCTCCCGCTCTCCCCGCGTCTCTCCTTCCCCGCGCGCCTCGCTGCCCT
TGATCTCTCCCGCAACGCCATCTCCGGCGCCGTCCCGCCGCGGATCGTCGCCGACCCCG
ACAACTCCGCTCTCCTCCTCCTCGACCTCTCCCACAACCGCTTCTCCGGCGAGATCCCC
GCCAGTATCGCAGCCGTACGGAGCCTGCAGGGGCTTTTTCTCGCGGACAACCAGCTTTC
CGGGGACATTCCTCCGGGGATAGGGAACCTGACCTATTTGCAGGTGCTGGATTTGTCGA
ATAACCGATTGTCCGGTTCAGTGCCTGCCGGACTTGCAGGCTGCTTCCAGCTTCTGTAC
CTGCAGCTTGGGGGTAACCAGCTCTCTGGGGCACTCCGTCCGGAGCTCGACGCACTAGC
TAGTCTCAAGGTTCTAGATTTGTCGAATAACAAGATATCTGGGGAGATTCCCCTGCCGC
TGGCTGGGTGCAGGTCTTTGGAGGTGGTGGACTTGTCAGGAAATGAGATCTCCGGTGAG

Figure 10

CTCAGCAGTGCTGTAGCGAAATGGCTGAGCTTGAAGTTCTTATCACTGGCTGGTAACCA
GCTCTCCGGCCACCCACCTGACTGGATGTTCTCGTTCCCCCTGCTCCAGTGGCTTGATT
TGTCTAGTAATAAGTTTGTGGGTTTCATCCCAGATGGGGGGTTCAATGTCAGTGAAGTG
CTTAACGGTGGAGGTGGTCAGGGGACTCCATCAGAGAGTGTGCTTCCACCCCAATTGTT
TGTGTCAGCTTCTGTGGACACGGTGTCATGGCAGTTGGATTTGGGGTATGATGTTCAGG
CAACTACTGGTATAGACCTGTCTGGGAATGAGCTCTGTGGGGAGATACCAGAAGGGTTG
GTTGACATGAAGGGGTTGGAGTATTTGAACCTCTCCTGTAATTACTTGGCTGGGCAGAT
CCCTGCGGGGCTTGGGGGCATGGGGAGGTTGCATACGCTTGACTTCTCACATAATAGGC
TGTCAGGGGAGGTGCCTCCTGGAATTGCAGCCATGACAGTGCTTGAGGTGCTTAACCTC
TCCTACAATAGCCTGTATGGGCCTTTGCCAACAACGAAGTTCCCAGGAGCATTAGCTGG
AAACCCAGGAATTTGCAGTGGGAAAGGGTGCTCTGAGAATGCAAGGACTCCAGAAGGGA
AAATGGAAGGTAGCAATCACCGCGGTTGGCTTGGTGGCTGGCATGGAGAGAATGGATGG
GTATCTCTTGGTGCATTTTGTATCAGCACAATGACTAGCTTCTATGTATCATTAGCAAC
CTTACTATGCTCCTCTAATGCAAGAAACTTCGTGTTTCGGCCTGTGAGGGTTGAATATT
AACAAGAGGGGAAATTGCAAAATCAGGTTGTTTTGAAGTTCGAGCGACTCTGGTCTGCA
GCTGATTAACAAGAAATATGAGCATATGAGATGGATATCTTCAGCCAAGAGGAAGTGCT
GTCTCTTTTAATGATCAATCAAGCTCTCTTGATTGTTTCCTAATATTCTTGATCTTGGG
ATGTGTAGATCTAGTTCTAATATTCCTACTGTTATAGAATGCAATCACCTGCTGGTGCT
TGGTTGTAGCCCTGGCGTGTTTGGAGGATTGGACACCAAGGATGCACATAATTTGAAGC
GCTGGTACTGTGAACCACTTCAGATGTAAATATTTTCTTTGGTTTTTAGTTCTGATCTA
GTTTAAAACTGGACATGTATTTAGTGTTGTTGAGCTACCTTTCGATGTTATATTATGTC
AATTTGCTGGAAAAAAAAAAAAAAAA

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